

REVIEW ARTICLE

Human gene mutations causing infertility

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The identification of gene mutations causing infertility in humans remains noticeably deficient at present. Although most males and females with infertility display normal pubertal development, nearly all of the gene mutations in humans have been characterised in people with deficient puberty and subsequent infertility. Gene mutations are arbitrarily categorised into four different compartments (I, hypothalamic; II, pituitary; III, gonadal; and IV, outflow tract). Diagnoses of infertility include hypogonadotrophic hypogonadism (compartments I and II), hypergonadotrophic hypogonadism (III), and obstructive disorders (compartment IV). Most gene mutations identified to date affect gonadal function, but it is also apparent that a large number of important genes in normal fertility have yet to be realised.

process because many different neuroendocrine factors, gonadal peptides, and growth factors are also involved in the process of normal reproduction.

COMPARTMENT I: THE HYPOTHALAMUS

Mutations of genes expressed in the hypothalamus generally result in hypogonadotrophic hypogonadism, a condition of absent or deficient puberty owing to low serum gonadotrophin, follicle stimulating hormone (FSH), and luteinising hormone (LH).^{1,2} Diminished gonadotrophin secretion results in reduced production of sex steroids (oestrogen in women, testosterone in men), which ultimately results in aberrant pubertal development. In these patients, the gonads are normal, but there is an impaired action of gonadotrophin releasing hormone (GnRH), the principle regulator of pituitary gonadotrophin function.^{1,2} Fertility is usually possible if pulsatile GnRH is administered, or more commonly, when exogenous gonadotrophins are given.

KAL1 gene

Males with Kallmann syndrome have X linked recessive idiopathic hypogonadotrophic hypogonadism (IHH) accompanied by anosmia caused by mutations in the *KAL1* gene, localised to the pseudoautosomal region of the Xp.^{3,4} The protein product of *KAL1*, anosmin, possesses neural cell adhesion molecule properties, and has homology to protease inhibitors, neurophysins, phosphatases, and kinases.^{3,4} A variety of *KAL1* gene mutations, including deletions and point mutations, in males with Kallmann syndrome proved causality.^{3–6} Anosmin provides a scaffold for GnRH neurones and olfactory nerves to migrate from the olfactory placode across the cribriform plate to synapse with mitral cells of the olfactory bulb. When anosmin is absent or defective, GnRH and olfactory neurones fail to synapse normally, resulting in Kallmann syndrome.

The *KAL1* gene escapes X inactivation and has an inactive pseudogene on Yq, but no comparable gene has been identified in rodents.⁶ *KAL1* has been characterised in chickens, with expression shown in the olfactory epithelium, oculomotor nucleus, Purkinje fibres of the cerebellum, midfacial mesenchyme, and meso- and metanephros.^{7,8} These patterns of expression have also been

Mutations in genes expressed in the hypothalamic-pituitary-gonadal-outflow tract axis cause pubertal and reproductive deficiencies in humans. In addition, there are a number of genes that have been shown to cause infertility in mice, but have not been studied yet in humans. To date, most mutations characterised in humans cause pubertal failure and infertility, while a few result in normal puberty, but cause infertility. All of the known mutations are shown in fig 1, but it is beyond the scope of this article to discuss all mutations in detail. The gene symbol can be used to access OMIM for a general review of specific mutations. Emphasis will be placed upon some of the more common gene mutations expressed in the hypothalamus, pituitary, gonad, and outflow tract. The identified mutations aid in our understanding of not only the pathophysiology of the reproductive deficiency, but also of normal reproductive function.

In an attempt to categorise mutations, they will arbitrarily be placed into compartments, which are useful in the endocrinological diagnosis of infertility (fig 1). The hypothalamus (compartment I) contains gonadotrophin releasing hormone (GnRH), which is the master switch that regulates the control of pituitary (compartment II) gonadotrophin production and secretion. The gonadotrophins, follicle stimulating hormone (FSH), and luteinising hormone (LH), in turn, stimulate the gonad (compartment III) to produce both sex steroids and gametes. The egress of the gametes from the "outflow tract" (compartment IV) facilitates the interaction and subsequent union of sperm and oocyte to form a zygote. Of course this overview of the compartments is an oversimplification of the reproductive

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Abbreviations: GnRH, gonadotrophin releasing hormone; IHH, idiopathic hypogonadotrophic hypogonadism; HH, hypogonadotrophic hypogonadism; AHC, adrenal hypoplasia congenita; POF, premature ovarian failure; APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; AR, androgen receptor

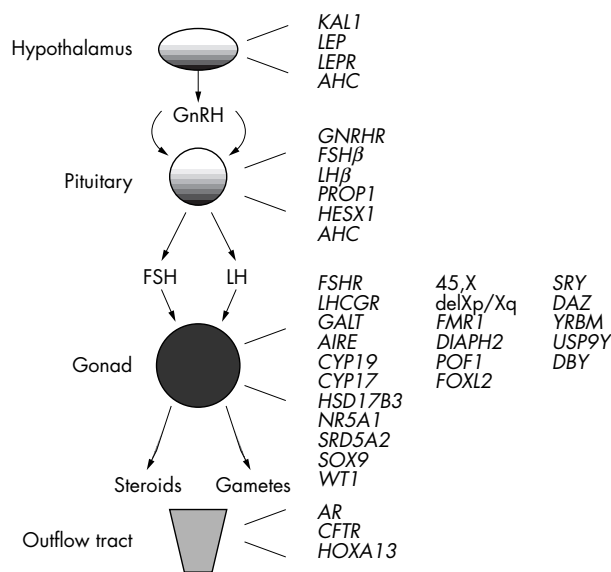


Figure 1 Each of the four compartments, hypothalamus, pituitary, gonad, and outflow tract, are shown, along with genes known to cause infertility. Gene symbols are provided in the text.

shown in human fetuses, which also express it in the cortico-spinal tract of the spinal cord.⁹⁻¹⁰ The patterns of *KAL1* expression explain the associated anomalies in some males with Kallmann syndrome such as synkinesia (mirror movements of extremities), visual abnormalities, renal agenesis, and midline facial clefting. About half of obvious X linked Kallmann syndrome families possess *KAL1* mutations and, of these, half also have unilateral renal agenesis.⁶ The frequency of *KAL1* mutations in unselected IHH males is considerably less (several percent), indicating that X linked inheritance is not the most common cause of human HH.¹¹ No *KAL1* gene mutations have been identified in females with IHH and anosmia, suggesting that other autosomal genes may be involved.

AHC gene

Males with adrenal hypoplasia congenita (AHC) display adrenal failure in infancy or during childhood owing to a failure of formation of the permanent zone of the adrenal gland. They have deficiency of both glucocorticoids and mineralocorticoids. Adequately treated AHC children fail to undergo puberty owing to HH, which may be accompanied by cryptorchidism. Mutations in the *AHC* gene have been shown to cause both AHC and HH.¹²⁻¹⁵ The *AHC* gene encodes a 470 amino acid protein, DAX1 (Dosage Sensitive Sex Reversal-AHC critical region of the X chromosome, gene 1). DAX1 is an orphan receptor (it has no known ligand) belonging to the steroid hormone superfamily, which is a transcription factor for the development of the pituitary gonadotrophs and adrenal cortex. It also appears to regulate gonadotrophin secretion at hypothalamic and pituitary sites.¹⁶

More than 50 *AHC* gene mutations have been described in patients with AHC/HH, most of which are nonsense and frameshift inactivating mutations throughout the gene.¹⁴⁻¹⁷ Missense mutations occur almost exclusively in the C-terminus. Although an X linked recessive disease producing gene, an *AHC* mutation was identified in a female with HH only (no adrenal disease), while the males of the family with the same mutation had both AHC and HH. In addition, *AHC* gene mutations were not identified in 100 IHH males without adrenal hypoplasia, suggesting that mutations cause AHC and HH.¹⁸

Initially, *AHC* was thought to be an ovarian determinant gene because of its localisation within the DSS region of the X

chromosome, a region of chromosome Xp which when duplicated is associated with sex reversal (feminisation) of 46,XY males. Conditional knockout studies, however, do not support its role as an ovarian determinant gene, but rather a role in spermatogenesis.¹⁹

LEP and LEPR genes

Leptin plays an important role in metabolism and in puberty. The leptin deficient *ob/ob* mice and leptin resistant *db/db* mice show obesity and hypogonadotrophic hypogonadism. Leptin (*LEP*) mutations have been found in a few families of obese subjects, who then have irreversible pubertal delay. Similar to the leptin deficient *ob/ob* mice, these patients had extreme obesity, hyperinsulinaemia, and hypogonadism, but, unlike the mice, they did not have stunted height, hyperglycaemia, or hypercortisolaemia.²⁰⁻²¹ A leptin receptor (*LEPR*) mutation produced a similar phenotype in a family with obesity and hypogonadotrophic hypogonadism, except for raised serum levels of leptin.²² These findings strongly implicate leptin action in the development of puberty.

COMPARTMENT II: THE PITUITARY

Mutations in pituitary genes causing infertility result either in deficiency of all or some of the pituitary hormones, thyroid stimulating hormone (TSH), prolactin, growth hormone, FSH, and LH. If isolated deficiencies of a single gonadotrophin occur, the phenotype is more restricted to impairment of pubertal development and fertility. In general, therapy for pituitary causes of infertility is very successful since the missing trophic factors can be replaced.

GNRHR gene

The gonadotrophin releasing hormone receptor (*GNRHR*), a G protein coupled receptor, represents the first autosomal recessive gene to possess mutations in human IHH. Surprisingly, no mutations had been identified in the GnRH ligand, despite the observation that the hypogonadal mouse had a GnRH gene deletion.²³⁻²⁶ Several investigators characterised gonadotrophin releasing hormone receptor (*GNRHR*) gene mutations.²⁷⁻²⁸ One group²⁷ hypothesised that partial loss of function mutations in *GNRHR* (as had been described in the *LH/hCGR* gene) could occur in patients with incomplete IHH (IHH patients with some evidence of puberty). Others²⁸ hypothesised that the *GNRHR* gene was a likely candidate gene for mutation in IHH patients because of the lack of *GnRH* mutations and the variability in response of IHH patients to exogenous GnRH. About ten different *GNRHR* mutations have been identified to date, and they are predominantly compound heterozygous missense mutations affecting binding and/or signal transduction.²⁷⁻³⁰ The phenotype of GnRH resistance ranges from complete IHH (no evidence of puberty or fertility) to incomplete (partial pubertal defects). Even in patients with complete IHH, GnRH administration may increase pituitary gonadotrophin responses. Although GnRH has been reported to result in pregnancy, it is likely that gonadotrophin therapy offers a better, more physiological treatment. The true prevalence of *GnRHR* mutations among all IHH patients is difficult to assess, but was 2.2% (1 of 46) of total IHH patients, and 7.1% (1 of 14) of patients in which an affected female was present.²⁸ Activating *GnRHR* mutations have not been described, but possible phenotypes could be precocious puberty, although none has been found in women with precocious puberty.³¹

Homeobox genes

EMX2 gene mutations result in schizencephaly (a defect in which cerebral hemispheres are separated by clefts), but have not been shown to cause IHH.³² It does show some similar patterns of expression to *KAL1*, since it is expressed in the olfactory bulbs and kidneys. In contrast, missense mutation in

the *HESX1* gene have been identified in some families with septo-optic dysplasia, a disorder characterised by panhypopituitarism, optic nerve atrophy, and other midline CNS abnormalities such as agenesis of the corpus callosum and septum pellucidum.³³ Both autosomal recessive³³ and autosomal dominant³⁴ mutations have been found in the *HESX1* gene. *Hesx1*, the mouse orthologue of *HESX1*, is expressed in the early forebrain, but later becomes restricted to Rathke's pouch, which ultimately becomes the anterior pituitary gland. Hypogonadotrophic hypogonadism, resulting from gonadotrophin deficiency, is a common feature of septo-optic dysplasia.

FSH β gene

FSH, along with LH, human chorionic gonadotrophin (hCG), and TSH comprise the pituitary glycoprotein hormones, although hCG is more highly expressed in the placenta. Each dimeric protein consists of a common α -subunit encoded by a single gene and a specific β -subunit. No human α -subunit mutations have been described, but similar to the α -subunit knockout mouse, the phenotype would be expected to include hypogonadotrophic hypogonadism and hypothyroidism. It is possible that α -subunit gene mutations could be lethal in humans, since humans would not have hCG (mice do not have hCG).

Female homozygous *FSH β* knockout mice had low serum FSH levels and were sterile because of arrested ovarian follicular development.³⁵ Surprisingly, serum oestradiol was normal in these mice despite unmeasurable serum FSH levels. Male homozygous *FSH β* knockout mice had normal levels of serum testosterone, small testes, and oligospermia but were fertile.³⁵ The phenotype of humans with *FSH β* mutations is similar, except that oestradiol levels are low.^{36–40} Affected females presented with absent or incomplete breast development, low FSH and oestradiol, high LH, and sterility.^{36–39} Despite the raised LH level similar to women with polycystic ovary syndrome (PCOS), hirsutism is not present suggesting that FSH might be necessary for normal LH induced androgen production by the theca cells.

Males with *FSH β* mutations present with azoospermia, but puberty may be normal or absent. Contrary to the *FSH β* knockout, humans with mutations suggested that FSH was essential for spermatogenesis in human males. In addition, more severe mutations affect androgen production, manifested clinically by delayed puberty and low testosterone.

LH β /hCG β genes

The *LH β /hCG β* gene complex, consisting of a single *LH β* gene and six *hCG β* genes, is polymorphic, but there is only one known human mutation in the *LH β* gene. The proband presented with pubertal delay, bilaterally small descended testes, low testosterone, and raised gonadotrophins. He responded to hCG administration, suggesting that the LH ligand might be defective. He was homozygous for a missense mutation of the *LH β* gene, which was detectable by immunoassay, but undetectable by radioreceptor assay.⁴¹

Although *LH β* polymorphisms have been identified, it is more problematical that they are definitive causes of LH dysfunction²; however, they may modify LH effects. No *LH β* mutations have been identified in females, but the phenotype would be expected to be normal pubertal development and anovulation without hirsutism.

PROPI gene

Mutations in *Prop1*, the mouse orthologue of human *PROPI*, cause the phenotype of the Ames dwarf mouse. Mutations of *PROPI* in humans cause combined pituitary hormone deficiency: growth hormone, TSH, prolactin, FSH, and LH.^{42–44} They present with short stature, hypothyroidism, and absent puberty. A variety of *PROPI* mutations have been identified

including missense mutations and deletions, but a particular 2 bp deletion is common, being a hotspot in the *PROPI* gene for mutations.^{42–45} In patients with IHH only, no *PROPI* mutations were identified, indicating that mutations generally affect several pituitary hormones.

COMPARTMENT III: THE GONAD

Gonadal causes of infertility constitute the largest group of disorders for which a molecular basis is known. Mutations affecting gonadal function include gonadotrophin receptors, steroid hormone receptors, steroid synthesis defects, as well as miscellaneous causes. Infertility caused by gonadal failure in general has a poor prognosis for treatment of the underlying condition. The best therapy usually involves the use of donor gametes (sperm or oocytes).

Chromosomal abnormalities

X chromosome

Whole X chromosome deletions

Women with a 45,X cell line, with or without mosaicism (46,XY, 46,XX, 47,XXX, or 46,X,iXq), generally manifest gonadal failure. About 90% of patients with a 45,X cell line (nearly all if the second line is 46,XY) present with the complete lack of pubertal development and no menarche.^{46–48} Typically, patients with a 45,X cell line have ovarian failure with irreversibly raised levels of gonadotrophins, FSH, and LH and low levels of oestradiol.^{46–48} Those 45,X patients that do attain normal puberty generally have ovarian failure before the age of 40.

The molecular basis of 45,X gonadal dysgenesis or Turner syndrome remains unknown, although it is likely that it is the result of haploinsufficiency of multiple genes on the X chromosome which affect ovarian function and stature.^{46–48} Classical Turner stigmata may be absent, but the most constant feature is short stature. A statural determinant gene (with a pseudogene on the Y chromosome), known as the pseudoautosomal homeobox containing osteogenic (*PHOG*) or short stature homeobox (*SHOX*) gene, is a transcription factor expressed in osteogenic cells that could be involved in the short stature of Turner syndrome.⁴⁹ *SHOX* mutations on one allele can cause Leri-Weill dyschondrosteosis, a skeletal dysplasia with disproportionate short stature, mesomelic limbs, and the Madelung deformity (sometimes seen in Turner syndrome).^{50–51} *SHOX* deletions on both X chromosomes have been identified in a female child with Langer mesomelic dysplasia, whose mother had a hemizygous deletion. Although deletions of the *SHOX* gene have been postulated to cause the short stature of Turner syndrome, there are no Turner patients to date with mutations in this gene.

X chromosome deletions

Deletions of portions of the X chromosome have been reported in a large number of patients, most of which are isolated. In general, deletions affecting Xp11 result in ovarian failure in about half of women, with menstrual function in the other half. Even in those with normal menstruation, fertility is rare. When the deletion on the X chromosome is more distal (Xp21 region), the phenotype is generally milder, although secondary amenorrhoea or infertility are common. Most women with Xp deletions are short, regardless of their ovarian function, indicating that other statural determinant genes probably lie within these regions.^{46–48–52}

Deletions involving the q arm of X generally result in ovarian failure if they involve the proposed critical region (the critical region hypothesis), that is, Xq13–q26. Although, nearly all patients with deletions within this region do have gonadal failure, exceptions do occur.⁵² Similar to short arm deletions, proximal (Xq13) deletions are usually more severe, with absent thelarche, primary amenorrhoea, and hypergonadotrophic hypogonadism occurring in most women. At the more

distal end of the long arm, menarche may occur, with or without ovarian failure. There have been reports of familial X chromosome deletions in which gonadal failure in females occurs. Puberty is normal so that these women achieve fertility, but then their menses cease before the age of 40 years. These familial ovarian failure syndromes seem to be inherited in an X linked dominant fashion. It has been suggested that there are two regions on the X chromosome that contain putative ovarian determinant genes, the POF1 region at Xq26-q28 and the POF2 region at Xq13.3-q22.⁵² Currently, there does not appear to be a single ovarian determinant gene in each region, but *DIAPH2* in the POF2 region may be one such gene (see below).

The molecular mechanism(s) responsible for gonadal failure with X chromosome deletions could involve the loss of a putative ovarian determinant gene(s) necessary to be present in two copies during ovarian development. Deleted ovarian determinant genes probably increase follicular atresia, but not to the extent of that observed in patients with a complete X chromosome deletion. It is also possible that partial X chromosome deletions might affect mitosis or meiosis, leading to enhanced follicular atresia.

X;autosome translocations

X;autosome translocations, although extremely rare, may affect reproductive capacity depending upon the location of breakpoints of the X chromosome. In a balanced X;autosome translocation, when one X is normal (Xn), and the other is an X;autosome translocation (Xt) chromosome, X inactivation is not usually random so that Xn is usually inactivated. If the translocated chromosome were inactivated (associated with XIST expression), the autosome would also be inactivated. Since monosomy for autosomes is lethal, the normal X chromosome must be inactivated to secure normality. In an unbalanced translocation involving the X chromosome, Xn is normally active while the X;autosome translocated product Xt containing XIST is inactivated in some cells.

Nearly all males and half of the females with X;autosome translocations are sterile.⁴⁸ In females, the phenotype of a balanced X;autosome translocation depends upon the position of the breakpoint and the functional status of the Xt chromosome. About three-quarters of patients will have an active Xt (and inactive Xn), while the others will have inactivation of Xt in some cells. In females with an active Xt in all cells and the breakpoint not interrupting any functional gene, about half have ovarian failure (breakpoints within the Xq13-q26 region) and the other half have a normal phenotype (breakpoints outside Xq13-q26).⁴⁸

The location of the X chromosome breakpoints involved in the translocation is evenly distributed when different studies are combined. The most common autosomes involved in X;autosome translocations are the acrocentric chromosomes 15, 21, and 22.⁴⁸ The pericentromeric regions of these autosomes appear particularly predisposed to pairing with the X chromosome. Translocations involving the X chromosome may cause ovarian failure by a position effect, the deletion of ovarian determinant genes, or by impairing the normal activity of the X chromosome in meiosis or mitosis, leading to accelerated atresia.

Single gene disorders of the X chromosome

Diaphanous gene

The last intron of the diaphanous 2 (*DIAPH2*) gene in the POF2 region was found to be disrupted in a woman with ovarian failure who had a balanced X;12 translocation, t(X;12)(q21;p1.3).⁵³ The *DIAPH2* gene on Xq22 is suspected to be an ovarian determinant gene since it has high homology to the *DIA* gene of *Drosophila*, which is expressed in the testes and ovary and causes sterility when mutations are present.⁵³ No point mutations have been described, so the importance of this gene in human gonadal development is unknown.

FMR1 gene

Fragile X syndrome is an X linked dominant disorder with incomplete penetrance characterised by mental deficiency, macro-orchidism (but normal testicular histology), and large ears and jaws in affected males. The *FMR1* gene, localised to the Xq27 fragile site, contains a triplet repeat of CGG bases which, when expanded to a premutation range, results in a carrier female. When carrier females have further meiotic expansion into the full mutation range, fragile X syndrome results. Some females with premutation alleles may be affected with mild degrees of mental deficiency or learning disability, but it has also been observed that female carriers may be at an increased risk of premature ovarian failure (POF), the cessation of menses before 40 years.

When women with sporadic POF are screened, approximately 3% will be premutation carriers.⁵⁴⁻⁵⁵ However, in families of women with POF, 12-15% may harbour *FMR1* premutation alleles.⁵⁴⁻⁵⁵ Interestingly, full mutations are rarely seen in POF women, suggesting that the premutation allele either causes or segregates with POF. *FMR1* is known to be expressed in the gonad, and although the exact mechanism is unknown, it is hypothesised that premutations of *FMR1* might affect ovarian development or function or both.⁵⁴⁻⁵⁵

Y chromosome

Decreased sperm parameters may consist of either oligospermia (less than 20 million/cc) or azoospermia (the absence of sperm), asthenospermia (less than 50% motility), and/or teratospermia (the presence of decreased percentages of normal sperm, less than 30% by World Health Organization or less than 4-6% by Strict Kruger morphology). Currently, most genes and chromosomal abnormalities affect sperm count or motility, but there certainly must exist gene mutations that affect sperm morphology.

Chromosomal disorders in men, including 47,XXY and 46,XX, generally result in azoospermia, but the pathophysiology is unclear. Some investigators have suggested that the presence of an additional X chromosome interferes with normal spermatogenesis. Balanced translocations have been identified in 1-2% of men with severe oligospermia and azoospermia. It is currently unknown if these translocations cause the oligospermia/azoospermia, but it is important to counsel these patients because of the increased risk of liveborn malformed children. X;autosomal translocations do produce azoospermia in most men, although the mechanism is unknown, but disruption of spermatogenesis genes on either chromosome is likely.

SRY gene

SRY (sex determining region on the Y chromosome) is located on the distal portion of Yp, adjacent to the more proximally located pseudoautosomal region. About 10-15% of patients with 46,XY gonadal dysgenesis (Swyer syndrome) possess mutations in this single exon gene.⁵⁶⁻⁵⁷ Most subjects with Swyer syndrome have complete absence of puberty, a normal vulva, vagina, and uterus, and streak gonads with the propensity for developing gonadoblastomas and germ cell tumours. Because the gonad is dysgenetic, antimüllerian hormone is deficient, explaining the presence of a uterus, as is testosterone, resulting in undermasculinisation of 46,XY males and subsequent infertility.

Spermatogenesis genes

The AZF (azoospermia factor) region on Yq11 is composed of three different regions AZFa, AZFb, AZFc, and recently a fourth (AZFd) has been suggested (fig 2). AZFa is the most proximal on Yq11, followed by AZFb, then AZFc (fig 2). Putative spermatogenesis genes in these regions include *USP9Y* (ubiquitin specific protease 9 on the Y chromosome) and *DBY* (dead box Y) on AZFa, *RBMV* (RNA binding motif on the Y

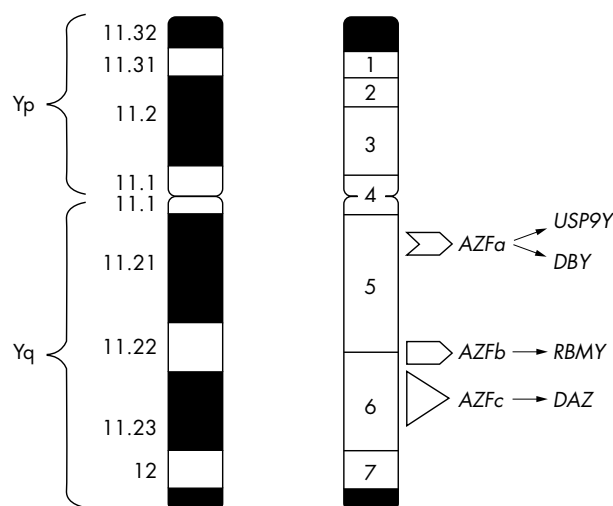


Figure 2 The Y chromosome is shown with G banding (left) and with deletion intervals 1-7 on the right. On the right, AZFa-c regions are shown, along with candidate spermatogenesis genes from each region.

chromosome) on AZFb, and DAZ (deleted in azoospermia) on AZFc.⁵⁸ DAZ was the first characterised putative spermatogenesis gene.⁵⁹

Although the exact number of copies is unknown, there appear to be four to six copies of *DAZ*, while the *RBMY* genes are part of a multigene complex of 20-50 genes/pseudogenes.⁵⁸ In contrast, *USP9Y* and *DBY* are single copy genes. Deletions of these regions of the Y chromosome (ascertained by studying a varying number of STSs, depending upon the study) have been reported to occur more frequently in infertile males, but have also been reported in fertile males. The only gene in the AZF region in which an intragenic mutation has been described is the *USP9Y* gene, also known as *DFFRY* (*Drosophila* Fat Facets related, Y linked) because of its homology to the corresponding gene in *Drosophila*.⁶⁰ A de novo 4 bp deletion in the splice donor site caused exon skipping and a resulting truncated protein. This mutation was absent in his fertile brother, suggesting that the *USP9Y* mutation caused spermatogenic failure. These investigators also identified a single gene deletion of *USP9Y* associated with spermatogenic failure by reanalysing a published study.

Of the more than 4800 published cases of infertile males screened for Y chromosome mutations, approximately 8.2% of infertile males and 0.4% of fertile males were found to have deletions of one or more AZF regions, although the prevalence ranges from 1-35% in individual studies.⁵⁸ In this review of published reports, of all Y chromosome deletions, AZFc was most common (60%), followed by AZFb (16%), then AZFa (5%). In the remainder, deletions of more than one of the regions were present (most commonly involving AZFc). Nearly all of the mutations were found in men with azoospermia (84%) or severe oligospermia (14%), defined as less than 5 million sperm/cc.⁵⁸

Interpretation of the data is extremely difficult because of (1) the presence of deletions in both infertile and fertile males, (2) the observation that the *DAZ* and *RBMY* genes are part of multigene clusters, (3) the different clinical phenotypes studied, (4) incomplete assembly of contigs on the Y chromosome, and (5) the lack of complete data on fertile control males. Recently, a double blind molecular study was published in which full endocrine and semen parameters were known for 138 consecutive men seen for intracytoplasmic sperm injection (ICSI) during in vitro fertilisation (IVF), 100 fertile men, and 107 young Danish military men.⁶¹ The men requiring ICSI represent the most severe male factor patients.

In their study, 21 STSs for AZFa-AZFc were studied. No deletions were identified in men with normal semen analyses or in any men with a count of more than 1 million/cc. Deletions of AZFc were identified in 17% of men with idiopathic azoospermia/cryptozoospermia (defined as less than 1 million sperm/cc) and 7% with non-idiopathic azoospermia/cryptozoospermia.⁶¹ Interestingly, no deletions of AZFa or AZFb were found in any of the men studied. These studies suggest that genes in the AZFc region may play a role in spermatogenesis, and represent the most common Y microdeletions identified in men with severe spermatogenic failure.

Autosomal disorders

FSHR gene

Homozygous *FSHR* KO mice have a similar phenotype to the *FSHβ* knockout mouse except that serum FSH levels are raised.⁶² Homozygous female *FSHR* knockout mice had low to normal oestradiol levels, immature follicles (primordial, primary, secondary), and sterility, while homozygous male *FSHR* knockout mice had decreased testicular size, low testosterone, and oligospermia. *FSHR* knockout males were fertile, although fertility was impaired and testosterone was low.

Human *FSHR* gene mutations have also been identified in humans, both in females⁶³⁻⁶⁴ and males.⁶⁵⁻⁶⁶ Females display hypergonadotrophic hypogonadism owing to FSH resistance⁶³⁻⁶⁴⁻⁶⁷ and the phenotype ranges from absent to normal breast development and primary amenorrhoea (Finnish Ala189Val mutation)⁶³⁻⁶⁷ to normal breast development and secondary amenorrhoea (compound heterozygous Ile160Thr/Arg573Cys).⁶⁴ Oestradiol levels range from low to normal, as does the level of ovarian follicular maturity.⁶³⁻⁶⁴⁻⁶⁷⁻⁶⁸

The prevalence of *FSHR* gene mutations has been addressed in several studies. The Finnish mutation (Ala189Val) is common in Finland (1% of females are heterozygotes),⁶³⁻⁶⁹ but was only seen in 1/1200 in Switzerland, 0/1100 in Denmark, and 0/540 in Singapore.⁶⁹ The Finnish allele was not identified in any of 35 North American females with premature ovarian failure.⁷⁰ Several *FSHR* polymorphisms have also been identified, but have not been shown to cause hypergonadotrophic hypogonadism (Liu, 1998, No 196; Conway, 1999, No 195) or polycystic ovary syndrome (Conway, 1999, No 195) in humans.

The Finnish Ala189Val mutation was also observed in males. Pubertal development and testosterone levels are normal, but the testes are small and there is a wide range of sperm concentrations, from very low to normal.⁶⁵ In general, the phenotypic effects of *FSHR* gene mutations do not seem to be as severe as those of the ligand FSHβ subunit. The functional studies of the Finnish mutations support these findings, since the mutant receptors have normal binding affinity, but are merely decreased in number.⁶³ Therefore, a raised FSH level may be able partly to compensate for impaired receptor function. Several *FSHR* polymorphisms have also been identified in males; however, they were present in both fertile and infertile men.⁷¹

One activating missense *FSHR* mutation has been reported in a male who underwent a trans-sphenoidal hypophysectomy for a pituitary adenoma. He maintained normal semen parameters despite low gonadotrophins postoperatively.⁶⁶ No family members were available, so the inheritance of this constitutively active *FSHR* could not be determined.

LHCGR gene

The LHCG receptor (*LHCGR*) is a G protein coupled receptor that binds both LH and hCG. Constitutive activation of the *LHCGR* gene causes familial male precocious puberty, inherited as an autosomal dominant trait. Interestingly, no known phenotype exists for females. However, it is the inactivating *LHCGR* mutations, which are autosomal recessive, that cause infertility in both males and females.

Inactivating *LHCGR* mutations in 46,XY subjects usually result in undermasculinisation with a blind ending vagina and absent uterus and fallopian tubes (mullerian inhibiting substance is normally produced). Occasionally, descended testes and a micropenis may occur. Serum levels of basal and hCG stimulated testosterone are low, basal LH levels are markedly raised (~20 mIU/ml), and FSH levels are normal.⁷² Germ cells are usually absent and Leydig cells are reduced.

The phenotype of 46,XX females with *LHCGR* mutations consists of normal sexual development and amenorrhoea.^{73,74} Serum LH may be normal to increased, FSH is normal, follicular phase oestradiol levels are normal, and progesterone is low. The uterus is small and the ovaries contain cysts. The phenotype was consistent with an anovulation, but with normal FSH function, she was capable of oestradiol synthesis. However, although LH levels are raised, hirsutism is not present and testosterone levels are normal. This suggests that although LH is the principle stimulator of androgen synthesis, perhaps some FSH is necessary.

Steroid enzyme genes (*CYP17*, *CYP19*, *HSD17B3*, and *SRD5A2*)

Mutations in several steroid enzyme pathway genes result in autosomal recessive infertility. The *CYP17* gene encoding the cytochrome P450 enzyme has both 17-hydroxylase activity (converting progesterone to 17-hydroxyprogesterone and pregnenolone to 17-hydroxypregnenolone) and carbon removing 17-20 desmolase activity (converting 17-hydroxypregnenolone to dehydroepiandrosterone (DHEA) and 17-hydroxyprogesterone to androstenedione). Therefore, androgen, progestin, and oestrogen deficiency results in both males and females. Cortisol is also deficient since it is derived from the precursor 17-hydroxyprogesterone; however, the mineralocorticoids 11-deoxycorticosterone (DOC) and corticosterone may be raised, with associated hypertension and hypokalaemic alkalosis.

The phenotype of *CYP17* mutations in 46,XX females consists of absent breast development, primary amenorrhoea, and raised gonadotrophins, resembling ovarian failure.⁷⁵ The vagina, uterus, and ovaries are present, but they are hypo-oestrogenic. 46,XY males have a similar phenotype except that they will not have a uterus or upper vagina since AMH is produced from their normal testes. Some males with partial deficiency may have sexual ambiguity. Many different *CYP17* mutations have been identified, with many being deletions and insertions.⁷⁵

The aromatase enzyme, encoded by the *CYP19* gene, converts the androgens testosterone and androstenedione to oestradiol and oestrone, respectively. 46,XX females are born with sexual ambiguity with clitoromegaly, but since they have ovaries, they do not have labioscrotal gonads.⁷⁶ At the time of puberty, FSH and LH rise and multicystic ovaries develop, but insufficient oestrogen is produced so that thelarche or

menarche do not occur. An interesting feature of aromatase deficiency is that the heterozygous mother carrying an affected female fetus can also display raised androgen levels and hirsutism.⁷⁶ This occurs because the fetal placenta (homozygous for *CYP19* mutations and derived from the fetus), which normally protects the fetus from hyperandrogenism, cannot successfully convert androgens to oestrogens. Although suspected to affect spermatogenesis in males, there is currently no conclusive evidence for this.⁷⁷ Mutations in the *17HSDB3* gene encoding 17-hydroxysteroid dehydrogenase type 3⁷⁸ and the *SRD5A2* gene encoding 5- α reductase type 2⁷⁹ cause undermasculinisation of 46,XY males, who also have infertility (fig 3).

Autoimmune regulatory (*AIRE*) gene

The autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is an autosomal recessive disorder in which multisystem autoimmune disease is observed. APECED, also known as autoimmune polyglandular syndrome type 1, is common in Finns, Iranian Jews, and Sardinians. Initial symptoms typically consist of moniliasis (60%), but endocrine abnormalities are also common: hypoparathyroidism (80%), adrenal failure (70%), ovarian failure (60%), and testicular failure (14%).^{80,81}

Mutations in the autoimmune regulator (*AIRE*) gene, a transcriptional factor with two PHD type zinc finger motifs, have been characterised in patients with APECED.^{80,81} The identification and characterisation of *AIRE* gene mutations represented the first report of a single gene defect causing systemic autoimmune disease in humans. About 80% of the mutations in Finns and Sardinians consist of a single nonsense mutation (Arg257X in Finns and Arg139X in Sardinians), while more than half of mutations in North American patients have a 13 bp deletion. At present, it is uncertain if specific mutations increase the likelihood of ovarian failure versus other endocrinopathies.

NR5A1 gene

The *FTZF1* gene, now known as the *NR5A1* gene, encodes for steroidogenic factor 1 (SF1) protein, a transcription factor important for steroidogenesis in the adrenal glands and gonads. Only two human mutations have been described in the *NR5A1* gene, one in a 46,XY male and the other in a 46,XX female. The first mutation, a heterozygous 2 bp deletion in an exon resulted in a phenotype of undermasculinisation of the 46,XY male and subsequent infertility.⁸² The proband presented as a phenotypic female with adrenal failure in neonatal life, who later had an impaired testosterone response to hCG administration suggesting defective gonadal function. At laparotomy, the proband had a normal uterus and bilateral streak gonads. Oestrogen therapy induced normal breast development, and she menstruated in response to combination oestrogen and progesterone preparations.⁸²

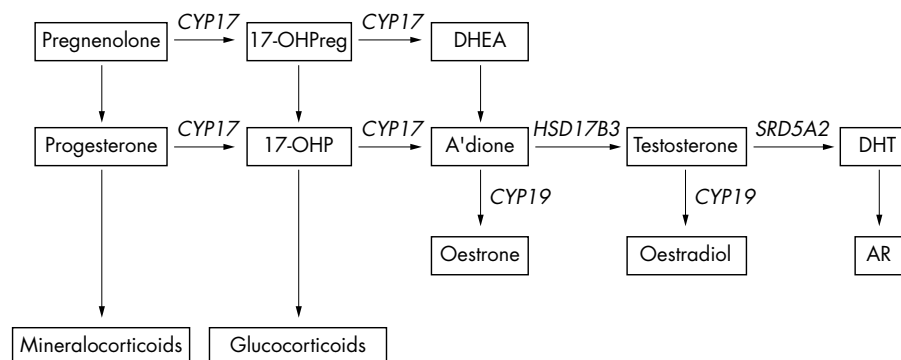


Figure 3 The steroid pathway is shown, illustrating the locations of gene defects. Only the enzymes for which there are gene defects are shown. 17-OHPreg = 17 hydroxypregnenolone; 17-OHP = 17 hydroxyprogesterone; A'dione = androstenedione; DHT = dihydrotestosterone.

The proband had a heterozygous point mutation of the *NR5A1* gene in the proximal (P) box of the first zinc finger, which eliminates SF1 from binding to DNA.⁸² The phenotype in genetic females would be expected to consist of adrenal failure, delayed puberty with absent breast development, and primary amenorrhoea, with raised gonadotrophins. A heterozygous point mutation was recently described in a 46,XX female, and no obvious phenotypic effects other than adrenal failure were noted.⁸³ However, since the endocrinological evaluation of this female has only been up to 27 months, it is unclear how puberty and reproduction will be affected.

GALT (galactose-1-phosphate uridylyltransferase) gene

Galactosaemia is an autosomal recessive disorder in which galactose cannot be converted to glucose. The phenotype includes failure to thrive, nausea and vomiting, hepatomegaly, cataracts, mental retardation, speech abnormalities, and haemolytic anaemia. A galactose free diet improves the prognosis for liver function and mental capacity, but not to normal. Several enzymes are involved in the process, but the *GALT* gene has profound sex specific effects upon reproduction. *GALT* is responsible for the conversion of galactose-1-phosphate and UDP-glucose to UDP-galactose and glucose-1-phosphate. Approximately two-thirds of women with *GALT* mutations have premature ovarian failure, but no men studied had testicular failure.⁸⁴ Affected females have normal pubertal development, but half present with primary amenorrhoea, while the other half have secondary amenorrhoea. Serum gonadotrophins are raised in affected females. The precise cause of ovarian failure is unknown, but could represent a detrimental metabolic defect during development caused by galactose-1-phosphate or abnormal glycosylation of gonadotrophin glycoproteins or their receptors.

FOXL2 gene

Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) is an autosomal dominant disorder, in which affected women with type I may have premature ovarian failure, while those with type II have the eyelid abnormalities only. Heterozygous mutations in *FOXL2* on chromosome 3q23 have been identified in these patients.⁸⁵ *FOXL2* is a winged helix/forkhead transcription factor that is expressed in the ovary, and it is likely that this gene is necessary for normal ovarian function.⁸⁵ It is unknown if *FOXL2* mutations occur in women with ovarian failure, who lack these characteristic eyelid and epicanthal abnormalities.

WT1 and SOX9 genes

Gene mutations in *WT1* and *SOX9* cause sexual ambiguity in 46,XY males, thereby producing infertility. Mutations in the *WT1* gene on 11p13 can cause either the Denys-Drash syndrome⁸⁶ or Frasier syndrome,⁸⁷ depending upon the type of mutation. The more severe Denys-Drash syndrome, caused by dominant negative mutations, is characterised by Wilms tumour, nephropathy, and sexual ambiguity in 46,XY males, with associated gonadoblastoma.⁸⁶ In contrast, Frasier syndrome, caused by heterozygous inactivating splicing mutations, is less severe and consists of Wilms tumour, sexual ambiguity, streak gonads, and renal failure.⁸⁷ Wilms tumour is much more common in patients with Denys-Drash syndrome compared with Frasier syndrome. *SOX9* mutations also produce autosomal dominant undermasculinisation of males and campomelic dysplasia.⁸⁸

COMPARTMENT IV: THE OUTFLOW TRACT

Androgen receptor (AR) gene

The androgen receptor (AR) belongs to the steroid superfamily of nuclear hormone receptors. The eight exon gene encodes for a protein containing an amino-terminus, a DNA binding domain, and a carboxy-terminal androgen binding

domain. 46,XY males with complete androgen insensitivity syndrome owing to mutations in the AR present as phenotypic females with primary amenorrhoea.⁸⁹ They have normal breast development, minimal axillary and pubic hair, and a blind vaginal pouch. The phenotype results from the absence of normal androgen interaction with its receptor. Since the testes are normal, antimüllerian hormone (AMH or MIS) is produced, which inhibits the formation of the uterus and upper vagina. The vagina appears as a blind vaginal pouch and no cervix is identified. The testes, which may be intra-abdominal or inguinal, are capable of making testosterone, and normal adult male levels are produced (300-1100 ng/dl). Incomplete forms of androgen insensitivity have also been described, and the phenotype is sexual ambiguity in males.⁸⁹

More than 300 different mutations (commonly missense mutations) in the AR gene have been reported to cause androgen insensitivity.⁹⁰ Of interest, nearly all of the exon 1 mutations cause complete or incomplete androgen insensitivity, and most of these produce a premature stop codon. However, most of the mutations causing androgen insensitivity occur in exons 2-8, despite the fact that exon 1 encodes for more than half of the protein.

The HOXA13 gene

To date, only one single gene disorder has been shown to affect uterine development in genetic females. Although this usually does not result in infertility, uterine anomalies may be associated with recurrent pregnancy loss. A nonsense mutation in the *HOXA13* homeobox gene predicted to reduce DNA binding has been identified in women with the hand-foot-genital syndrome.⁹¹ Patients with this autosomal dominant disorder typically have small hands and feet, along with a duplication of the uterus. Specific hand anomalies include short first metacarpals, short middle phalanges of the fifth fingers, small distal phalanges of the thumbs, and delayed ossification or fusion of wrist bones. The big toe has a short first metatarsal and a small, pointed distal phalanx. Uterine anomalies typically include bicornuate (two uterine horns, one cervix) or didelphic (two separate uterine horns and two cervices) uteri. Urinary anomalies, including displaced urethral or ureteral openings, are also associated with hand-foot-genital syndrome. Males may have varying degrees of hypospadias. Although congenital absence of the uterus and vagina seems like another potential phenotype of *HOXA13* gene mutations, none has been described in these patients to date.

CFTR gene

Congenital bilateral absence of the vas deferens occurs in about 1% of infertile males. Probably at least 80-90% of these patients are compound heterozygotes for mutations in the *CFTR* gene.⁹²⁻⁹³ Since these males usually have normal testicular sperm, they may have an epididymal or testicular aspiration to retrieve sperm. Since these sperm are not motile, intracytoplasmic injection of oocytes in a cycle of in vitro fertilisation results in excellent pregnancy rates. Therefore, it is extremely important that female partners be screened for *CFTR* mutations as well.

CONCLUSIONS

At present, there are a modest number of gene mutations known to cause infertility in humans. Most of these disrupt normal puberty and subsequently cause infertility. However, with the completion of the draft of the human genome project, the number of mutations described within the next five years will increase exponentially. These newly discovered gene mutations will increase our understanding of normal reproductive physiology, and will likely lead to improved infertility treatments.

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